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Two-Step Assembly Dynamics of the *Bacillus subtilis* Divisome

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Supplementary information

Table S1: Delay between EzrA and DivIVA localization in vegetative cell cycles

Cell cycle ^a length (min)	Delay EzrA-DivIVA ^b (%)	Cell cycles ^c (No.)
40	36.8	26
50	37.4	43
60	39.0	52
70	30.6	29
80-110	43.3	50

^a Total length of the cell cycle as determined by membrane staining. Fast dividing cells exhibit a short cell cycle length, while slow dividing cells have a long cell cycle length.

^b Delay between EzrA-GFP and DivIVA-GFP localization, expressed as a percentage of the progression within the cell cycle and calculated from the averages of the EzrA-GFP and DivIVA-GFP localization times in each group.

^c Number of cell cycles analysed for each category, expressed as the sum of the EzrA-GFP and DivIVA-GFP cell cycles considered.

Figure S1: Vector pPG2

Individual cloning sites are highlighted by an asterisk.

Figure S2: Assembly initiation times of EzrA-GFP and DivIVA-GFP in vegetative cell cycles.

EzrA-GFP and DivIVA-GFP assembly initiation times expressed as a percentage of the cell cycle progression. Single-cell values for both strains were plotted together. ◆ = EzrA-GFP, ○ = DivIVA-GFP.

Movie S1

Growing microcolonies of EzrA-GFP and DivIVA-GFP strain. The film shows 350 min (35 frames) of the growth of several microcolonies of strains EzrA-GFP and DivIVA-

GFP. Phase contrast, FM5-95 and GFP images were taken every 10 minutes. The overlay of the two fluorescence channels is shown. Spores of the two strains were mixed in an equal ratio and germinated as described in Materials and Methods. DivIVA-GFP strain is easily distinguishable for the polar localization of DivIVA-GFP. The data derived from this film was used for Figs. 4, 5, S2 and Table S1.

Movie S2

Growing microcolonies of GFP-FtsL strain. The film shows 585 min (45 frames) of the growth of several microcolonies. Phase contrast, FM5-95 and GFP images were taken every 13 minutes. Spores were germinated as described in Materials and Methods, with the addition of 0.3% xylose. The overlay of the two fluorescence channels is shown. The data derived from this film was used for Fig. 5.